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A Geno Technology, Inc. (USA) brand name

HOOK™ HRP SULFO Labeling Kit

For Coupling HRP to Proteins, via Sulfhydryls
Maleimide activated HRP conjugation kit

(Cat. # 786-1639, 786-1640)

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INTRODUCTION

The HOOK™ HRP SULFO labeling kit is an efficient enzyme labeling kit for tagging proteins with horseradish peroxidase (HRP). The kit contains activated HRP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The HRP has been maleimide-activated using Sulfo-SMCC, a hetero bifuncational reagent that contains an N-hydroxysuccinimide ester and a maleimide group. The activated HRP saves time since the first step of the normal two steps maleimide activation procedure is already complete.

The kit is supplied with two modification reagents SATA (*N*-Succinimidyl S-acetylthioacetate) and 2-mercaptoethylamine·HCl (2-MEA) which provides the researcher with choice for producing free sulfhydryl groups on proteins which are to be used in conjugation with HRP. SATA adds free sulfhydryls to existing amine groups of proteins, and mercaptoethylamine·HCl, a mild reducing agent reduces IgG to give IgG fragments with low avidity but intact affinity to antigen.

The HOOK™ HRP SULFO labeling kit contains reagents sufficient to carry our five 1 mg antibody labeling reactions.

ITEM(S) SUPPLIED

Description	**Cat. # 786-1640**	**Cat. # 786-1639**
HOOK™ HRP SULFO	5 x 1mg	5 x 1mg
Optimizer Buffer™ III [5X]	2 x 25ml	-
SATA	10mg	-
2-mercaptoethylamine·HCl	50mg	-
DMF	2ml	-
Hydroxylamine.HCl	50mg	-
SpinOUT™GT-600, 5 ml	5 Columns	

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, immediately remove HOOK™ HRP SULFO and SATA and store at -20°C protected from moisture. Store SpinOUT™GT-600, 5 ml columns and Optimizer Buffer™ III [5X] at 4°C. Store other components at room temperature.

IMPORTANT INFORMATION

- Bring all the kit components to room temperature before use.

- To prepare IgG for conjugation to HOOK™ HRP SULFO, one must produce free sulfhydryl groups on IgG. One strategy is to reduce native disulfide bonds in the antibody using 2-MEA. This will selectively cleave between heavy chains of IgG to produce monovalent antibody with free sulfhydryl group for HRP conjugation. This method keeps the antigen binding site intact, but the antibody avidity is lowered. The second strategy is to add sulfhydryl using SATA. SATA reacts with primary amines present on side-chains of lysine residues to produce protected sulfhydryl groups which are deprotected using hydroxylamine.HCl. This method does not fragment antibody; however, it may affect antigen binding site in case that site has many lysine residues. Depending upon your antibody or protein one can choose between these two methods.
- Maleimide groups (in HOOK™ HRP SULFO) reacts with sulfhydryl groups at pH 6.5-7.5 to form stable thioether bond. At pH >7.5 reactivity towards primary amine or hydrolysis of maleimide group can occur.
- Removes excess 2-MEA (reducing agent) or hydroxylamine (deacetylation agent) before conjugating antibody with HOOK™ HRP SULFO (check protocol for detail).

PREPARATION BEFORE USE

Optimizer Buffer™ III [5X]: Supplied as a 5X solution. Mix with 4 volumes distilled water. For 100ml, add 20ml Optimizer Buffer™ III to 80ml distilled water.

PROTOCOL

Immunoglobulin G (IgG) preparation for conjugation with HOOK™ HRP SULFO

Selective Reduction of IgG

1. Weigh out 1.5mg 2-mercaptoethylamine and add to 1ml of 1mg/ml IgG solution.
2. Dissolve with gentle pipetting.
3. Incubate the tube at 37°C for 90 minutes.

Removal 2-mercaptoethylamine using SpinOUT™ GT-600, 5ml column

1. Centrifuge the SpinOUT™ GT-600 column at 1,000g for 2 minutes to compact the resin.
2. Prepare the Spin-*OUT*™ GT-600 column by removing the top and then bottom caps. Place into an appropriate collection tube.
3. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
4. Place the column in a new collection tube and remove the cap.
5. Add 10 ml of 1 X Optimizer Buffer™ III to the center of column.
6. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
7. Repeat steps 5 and 6 two more times, ensuring the buffer is discarded after each centrifugation.
8. Centrifuge the column at 1,000 g for 2 minutes to remove residual buffer
9. Place the column in a new collection tube and remove the cap.
10. Slowly, apply 1 ml of reduced IgG solution to the center of column.

11. Centrifuge the column at 1,000g for 4 minutes to collect the reduced antibody solution.
12. Use the reduced antibody for conjugation immediately for conjugation.

Addition of Sulfhydryls with SATA

If your protein or peptide lacks free sulfhydryls or have very few, additional free sulfhydryl groups can be added with the use of SATA.

1. Weigh out 2mg SATA into a clean tube and immediately before use dissolve in 200μl DMF.
2. Add 4μl SATA solution to 1ml (1mg/ml) IgG solution to give a 25molar excess of SATA.
3. Mix SATA with antibody solution and incubate at room temperature for 30 minutes.
4. The modified IgG solution is stable and can be stored at -20°C.

Deacetylation of SATA treated antibody with hydroxylamine to unmask sulfhydryl groups for conjugation.

1. Weigh out 2mg hydroxylamine into a clean tube and immediately before use add 100μl 1X Optimizer Buffer™ III to make the deacetylation solution.
2. Add 50μl deacetylation solution to the IgG solution and incubate for 2 hours at room temperature.

Removal of hydroxylamine using SpinOUT™ GT-600, 5ml column

1. Centrifuge the SpinOUT™ GT-600 column at 1,000g for 2 minutes to compact the resin.
2. Prepare the Spin-*OUT*™ GT-600 column by removing the top and then bottom caps. Place into an appropriate collection tube.
3. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
4. Place the column in a new collection tube and remove the cap.
5. Add 10 ml of 1 X Optimizer Buffer™ III to the centre of column.
6. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
7. Repeat steps 5 and 6 two more times, ensuring the buffer is discarded after each centrifugation.
8. Centrifuge the column at 1,000 g for 2 minutes to remove residual buffer
9. Place the column in a new collection tube and remove the cap.
10. Slowly, apply 1 ml of deacetylation solution treated antibody solution to the center of column.
11. Centrifuge the column at 1,000g for 4 minutes to collect the modified antibody solution free of hydroxylamine.
12. Use the modified antibody for conjugation immediately for conjugation.

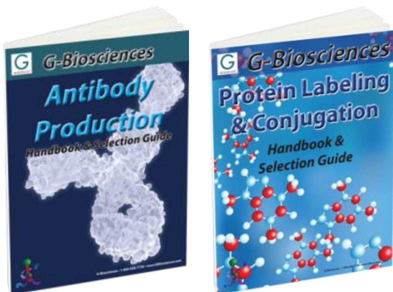
Conjugation of HRP to IgG

The following protocols are designed for the conjugation of HRP to IgG molecules, but they can be adapted for use with other proteins, peptides or ligands.

1. Dissolve the HOOK™ HRP SULFO in 1ml (1mg/ml) modified or reduced IgG.
2. Incubate at room temperature for 1 hour with gently tumbling. Alternatively increase incubation up to 12 hours as this may lead to increased conjugation efficiency.
3. The conjugated antibody is now ready for use. For long-term storage, remove the EDTA (present in Optimizer™ buffer III) by dialysis (Tube-O-DIALYZER™, Cat. #786-610 -786-624) or desalting column (SpinOut™GT-600 column, Cat. # 786-704) equilibrated with PBS buffer (Cat. #786-377).
4. Add glycerol to a final concentration of 50% and store at -20°C.

RELATED PRODUCTS

Download our Antibody Production and Protein Labeling & Conjugation Handbooks



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